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| (54) Title: METHOD CAPABLE OF INCREASING COMPETENCY OF BACTERIAL CELL TRANSFORMATION | | | |
| (57) Abstract The invention concerns bacterial strains capable of enhanced transformation efficiencies that are produced by the introduction of the F' genetic material. The invention also concerns processes for producing transformable competent bacteria with enhanced transformation efficiencies. | | | |

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TITLE OF THE INVENTION:METHOD CAPABLE OF INCREASING COMPETENCY OF BACTERIAL
CELL TRANSFORMATIONCROSS REFERENCE TO RELATED APPLICATIONS

5 This application is based upon and claims priority from United States Provisional Application No: 60/011,040.

FIELD OF THE INVENTION:

10 The invention relates to improved bacteria, particularly *Escherichia coli* (*E. coli*) bacteria capable of high transformation efficiencies, methods for producing improved bacterial strains capable of high transformation efficiencies, and methods for obtaining high transformation efficiencies with bacteria, particularly *E. coli* bacteria. Specifically, it relates to methods of producing and using bacteria, particularly *E. coli* bacteria that contain F' episome genetic material and are capable of exhibiting enhanced
15 transformation efficiencies.

BACKGROUND OF THE INVENTION:

20 High efficiency chemically competent *E. coli* bacteria (bacterial cells that can be transformed with DNA) are used extensively in the generation of cDNA libraries and the cloning of samples containing small amounts of target sequences. The ability to generate representative cDNA libraries, one in which each mRNA species present in the subject cell is represented in the library, relies on many factors. One of the major factors determining the quality of a cDNA library is the number of clones represented in the library. Using competent bacteria having a high transformation efficiency
25 increases the probability of obtaining rare, under-represented clones in plasmid libraries. Also, when cloning samples containing small amounts of target DNA or cloning the DNA products of complex DNA

manipulations such as the DNA products of single or multiple blunt ended ligations, the use of high efficiency bacteria is essential.

Early attempts to achieve transformation of *E. coli* were unsuccessful and it was generally believed that *E. coli* was refractory to transformation. However, Mandel and Higa (*J. Mol. Bio.* 53: 159-162 (1970)) found that treatment with CaCl_2 allowed *E. coli* bacteria to take up DNA from bacteriophage λ . In 1972, Cohen *et al.* showed CaCl_2 -treated *E. coli* bacteria were effective recipients for plasmid DNA (Cohen *et al.*, *Proc. Natl. Acad. Sci.*, 69: 2110-2114 (1972)). Since transformation of *E. coli* is an essential step or cornerstone in many cloning experiments, it is desirable that it be as efficient as possible (Lui and Rashidbaigi, *BioTechniques* 8: 21-25 (1990)). Several groups of workers have examined the factors affecting the efficiency of transformation.

Hanahan (*J. Mol. Biol.* 166: 557-580 (1983), herein incorporated by reference) examined factors that affect the efficiency of transformation, and devised a set of conditions for optimal efficiency (expressed as transformants per μg of DNA added) applicable to most *E. coli* K12 strains. Typically, efficiencies of 10^7 to 10^9 transformants/ μg can be achieved depending on the strain of *E. coli* and the method used (Liu & Rashidbaigi, *BioTechniques* 8: 21-25 (1990), herein incorporated by reference).

Many methods for bacterial transformation are based on the observations of Mandel and Higa (*J. Mol. Bio.* 53: 159-162 (1970)). Apparently, Mandel and Higa's treatment induces a transient state of "competence" in the recipient bacteria, during which they are able to take up DNAs derived from a variety of sources. Many variations of this basic technique have since been described, often directed toward optimizing the efficiency of transformation of different bacterial strains by plasmids. Bacteria treated according to the original protocol of Mandel and Higa yield 10^5 - 10^6 transformed colonies/ μg of supercoiled plasmid DNA. This efficiency can be enhanced 100- to 1000-fold by using improved strains of

E. coli (Kushner, In: *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering*, Elsevier, Amsterdam, pp. 17-23 (1978); Norgard *et al.*, *Gene* 3:279-292 (1978); Hanahan, *J. Mol. Biol.* 166: 557-580 (1983)) combinations of divalent cations ((Kushner, In: *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering*, Elsevier, Amsterdam, pp. 17-23 (1978)) for longer periods of time (Dagert and Ehrlich, *Gene* 6: 23-28 (1979)) and treating the bacteria with DMSO (Kushner, In: *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering*, Elsevier, Amsterdam, pp. 17-23 (1978)), reducing agents, and hexamminecobalt chloride (Hanahan (*J. Mol. Biol.* 166: 557-580 (1983)).

Incubation of *E. coli* in solutions that contain multivalent cations is an important step in the transformation of *E. coli*. A number of multivalent cations are capable of affecting DNA transformation of *E. coli*. In addition to calcium cations, manganese, magnesium and barium cations can affect DNA transformation of *E. coli* and the use of manganese or barium cations rather than calcium cations has lead to higher transformation efficiencies with some strains of *E. coli* (Taketo, *Z. Naturforsch Sect. C* 30: 520-522 (1975); Taketo, *Z. Naturforsch Sect. C* 32: 429-433 (1975); Taketo & Kuno, *J. Biochem.* 75: 895-904 (1975)). A variety of other compounds affect transformation efficiencies. Organic solvents and sulhydryl reagents can also influence transformation efficiencies (Hanahan (*J. Mol. Biol.* 166: 557-580 (1983); Kushner, In: *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering*, Elsevier, Amsterdam, pp. 17-23 (1978); Jessee, J.A. and Bloom, F.R., U.S. Patent No. 4,981,797 (1991)).

Incubation of *E. coli* at temperatures around 0°C, often on ice, in buffers containing multivalent cations is an important step in the production or generation of competent cells of *E. coli*. A rapid heat shock or temperature transition after incubation of the *E. coli* with target DNA further improves transformation efficiencies (Mandel and Higrá, (*J. Mol. Bio.* 53: 159-162 (1970)). Typically, the solutions containing *E. coli* and

target DNA are transferred from 0°C to temperatures between 37 and 42°C for 30 to 120 seconds. The temperature at which *E. coli* bacteria are grown prior to incubation at 0°C can also affect transformation efficiency. Growing *E. coli* bacteria at temperatures between 25 and 30°C can improve the transformation efficiency of *E. coli* bacteria compared with *E. coli* bacteria grown at 37°C (Jessee, J.A. and Bloom, F.R., U.S. Patent No. 4,981,797 (1991)). *E. coli* bacteria that are grown at temperatures between 25 and 30°C, in contrast to 37°C, may require a heat shock at less than 37 to 42°C, or a heat shock of a shorter duration, for optimal results (Jesse and Bloom, U.S. Patent No. 4,981,797 (1991); Inoue *et al.* *Gene* 96:23-28 (1990)).

Transformation efficiency can be affected by the *E. coli* strain used. The selection of an *E. coli* strain that is capable of high transformation with the specific competence protocol adopted is an important step in the development of a procedure to produce *E. coli* bacteria capable of high transformation efficiencies. Different strains can exhibit different transformation efficiencies depending on the competence protocol used. Lui and Rashidbaigi, *BioTechniques* 8: 21-25 (1990), compared the transformation efficiency of five *E. coli* strains, HB101, RR1, DH1, SCS1 and JV30 and showed that the transformation efficiencies of these strains varied according to the methodology adopted.

A number of procedures exist for the preparation of competent bacteria and the introduction of DNA into those bacteria. A very simple, moderately efficient transformation procedure for use with *E. coli* involves re-suspending log-phase bacteria in ice-cold 50 mM calcium chloride at about 10^{10} bacteria/ml and keeping them ice-cold for about 30 min. Plasmid DNA (0.1 mg) is then added to a small aliquot (0.2ml) of these now competent bacteria, and the incubation on ice continued for a further 30 min, followed by a heat shock of 2 min at 42°C. The bacteria are then usually transferred to nutrient medium and incubated for some time (30 min to 1 hour) to allow phenotypic properties conferred by the plasmid to be expressed, e.g. antibiotic resistance commonly used as a selectable

marker for plasmid-containing cells. Protocols for the production of high efficiency competent bacteria have also been described and many of those protocols are based on the protocols described by Hanahan (*J. Mol. Biol.* 166: 557-580 (1983)).

5 The F episome is a genetic element that may exist as a free genetic element or become integrated into the bacterial genome. The presence of the F episome, whether in a free or integrated form, has important consequences for the host bacterium. F-positive bacteria exhibit surface
10 appendages called pilli, which provide attachment sites that facilitate the infection of certain RNA and single-stranded DNA viruses. Many *E. coli* strains have been constructed to contain an F plasmid in order to facilitate the infection of those strains by single-stranded DNA viruses. *E. coli* strains engineered for this purpose include: JM101 (Messing, In *Recombinant DNA: Proceedings of the Third Cleveland Series on Macromolecules*,
15 Elsevier, Amsterdam p 143-153 (1981)); JM105, JM107, JM109 and JM110 (Yanish-Perron *et al.*, *Gene* 33: 103-119 (1985)); TG1 (Gibson, Ph.D. Thesis, Cambridge University, England (1984)); TG2 (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor p. 4.14 (1989)); XL1-Blue (Bullock *et al.*, *BioTechniques* 5.4:376-378 (1987)); XS127 and XS101 (Levinson
20 *et al.*, *Mol. Appl. Genet.* 2:507-517 (1984)); 71/18 (Dente *et al.*, *Nucleic Acids Res.* 11: 1645-1655 (1983)); KK2186 (Zagursky and Berman, *Gene* 27:183-191 (1984)); and MV1184 (Viera and Messing, *Methods Enzymol.* 153: 3-11 (1987)).

25 Transformation efficiency was not thought to be enhanced by the addition of F' episome genetic material. (Hanahan (*J. Mol. Biol.* 166: 557-580 (1983); Bullock *et al.* (1987)). Indeed, the addition of a F' episome to the *E. coli* strain AG1 produced an *E. coli* strain (XL1-Blue) with a reduced transformation efficiency (Bullock *et al.* (1987)). Contrary to this background, Applicants' invention involves the use of F' genetic material
30 to provide modified *E. coli* having improved transformation efficiency compared with *E. coli* without F' genetic material.

Another rapid and simple method for introducing genetic material into bacteria is electroporation (Potter, *Anal. Biochem.* 174: 361-73 (1988)). This technique is based upon the original observation by Zimmerman *et al.*, *J. Membr. Biol.* 67: 165-82 (1983), that high-voltage electric pulses can induce
5 cell plasma membranes to fuse. Subsequently, it was found that when subjected to electric shock (typically a brief exposure to a voltage gradient of 4000-16000 V/cm), the bacteria take up exogenous DNA from the suspending solution, apparently through holes momentarily created in the plasma membrane. A proportion of these bacteria become stably
10 transformed and can be selected if a suitable marker gene is carried on the transforming DNA transformed (Newman *et al.*, *Mol. Gen. Genetics* 197: 195-204 (1982)). With *E. coli*, electroporation has been found to give plasmid transformation efficiencies of 10^9 - 10^{10} / μ g DNA (Dower *et al.*, *Nucleic Acids Res.* 16: 6127-6145 (1988)).

15 Bacterial cells are also susceptible to transformation by liposomes (Old and Primrose, In *Principles of Gene Manipulation: An Introduction to Gene Manipulation*, Blackwell Science (1995)). A simple transformation system has been developed which makes use of liposomes prepared from cationic lipid (Old and Primrose, (1995)). Small unilamellar (single bilayer)
20 vesicles are produced. DNA in solution spontaneously and efficiently complexes with these liposomes (in contrast to previously employed liposome encapsidation procedures involving non-ionic lipids). The positively-charged liposomes not only complex with DNA, but also bind to bacteria and are efficient in transforming them, probably by fusion with the
25 cells. The use of liposomes as a transformation or transfection system is called lipofection.

SUMMARY OF THE INVENTION:

The present invention provides novel bacterium, particularly novel
30 *E. coli* bacterium, capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of F' episome

genetic material. The invention also concerns methods for the use of novel bacteria, particularly novel *E. coli* bacteria, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. Furthermore, the invention also provides methods for constructing bacteria capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. Indeed, the invention may be used for the insertion of exogenous DNA sequences from other *E. coli* bacteria or other organisms into the novel bacteria of the present invention.

DETAILED DESCRIPTION:

One object of the present invention is to provide a bacterium containing F' genetic material capable of an enhanced transformation efficiency. A particular object of the present invention is to provide an *E. coli* bacterium containing F' genetic material capable of an enhanced transformation efficiency.

A further object of the present invention is to provide a process for producing an Enterobacteriaceae (especially an *E. coli* bacterium) containing F' genetic material capable of an enhanced transformation efficiency, comprising the following steps: (a) introducing F' genetic material into a bacterium; (b) selecting the bacterium containing F' genetic material; and (c) recovering the bacterium containing F' genetic material.

Another object of the present invention is to provide a process for preparing competent bacteria comprising the following steps: (a) growing a bacterium (especially an *E. coli* bacterium) containing F' genetic material capable of an enhanced transformation efficiency in a growth-conductive medium; and (b) rendering the bacterium competent.

The present invention further concerns a novel bacterium, especially an Enterobacteriaceae, and particularly a novel *E. coli* bacterium capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. The present

invention also concerns processes for transforming *E. coli* bacterium containing F' genetic material that are capable of enhanced transformation efficiencies.

5 The present invention pertains to bacteria capable of high efficiency transformation. Such bacteria may be any bacteria whose efficiency of transformation can be enhanced by the introduction of F' episome genetic material. Examples of suitable bacteria include bacteria of the Enterobacteriaceae, and in particular, bacteria of the genera *Escherichia*, *Salmonella*, especially *E. coli* and *Salmonella* species. In a preferred
10 embodiment, the bacterium of the present invention may be any *E. coli* strain capable of high efficiency transformation whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. In a further preferred embodiment of the present invention, the bacterium may be any *E. coli* K strain or derivative or equivalent thereof.
15 As used herein, a "derivative" of a bacterium is any bacterium that results from any alteration (or series or alterations), naturally occurring or otherwise, of that bacterium. As used herein, an "equivalent" of a bacterium is any bacterium that has a transformation efficiency (as measured by transformants/ μ g DNA added) that is equivalent to the
20 transformation efficiency of that bacterium. In an even more preferred embodiment, the bacterium of the present invention will preferably be a derivative of *E. coli* K, such as MM294 (Meselson & Yuan, *Nature* 217: 1110-1114 (1968), or a derivative or equivalent thereof. In a further even more preferred embodiment, the bacterium of the present invention will be
25 preferably be *E. coli* DH5 α or a derivative or equivalent thereof, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. In the most preferred embodiment, the bacterium of the present invention will be more preferably be *E. coli* DH5 α , whose efficiency of transformation is enhanced by the introduction of F' episome genetic
30 material.

Transformation, in the context of the current invention, is the process by which exogenous DNA is inserted into a bacterium, causing the bacterium to change its genotype and/or phenotype. Such a change in genotype or phenotype may be transient or otherwise. Exogenous DNA is any DNA, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism. Preferably, exogenous DNA is any DNA, whether naturally occurring or otherwise, from any source that is capable of being inserted into bacteria. More preferably, exogenous DNA is any DNA, whether naturally occurring or otherwise, from any source that is capable of being inserted into DH5 α . Even more preferably, exogenous DNA is any DNA, whether naturally occurring or otherwise, from any source that is capable of being inserted into DH5 α bacteria capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. Such exogenous DNA includes, without limitation, plasmid DNA and lambda DNA.

The bacteria of the present invention are capable of acting as a recipient for inserted exogenous DNA. In a preferred embodiment, bacteria capable of acting as a recipient for exogenous DNA are prepared by inoculating medium which supports their growth. In a more preferred embodiment, *E. coli* DH5 α bacteria containing F' genetic material capable of an enhanced transformation efficiency are grown at 28°C in SOB media (20 g bacto-trytone, 5 g bacto-yeast extract, 5 g NaCl, per liter, 2.5 mM KCl, 10 mM MgCl₂ equilibrated to pH 7.0 with NaOH) media containing additional multivalent cations until the optical density of the solution was 0.3 O.D.₅₅₀. In an even more preferred embodiment, *E. coli* DH5 α bacteria containing F' genetic material capable of an enhanced transformation efficiency are grown at 28°C in SOB media containing 20mM magnesium cations until the optical density of the solution is 0.3 O.D.₅₅₀.

In a preferred embodiment, the bacteria are harvested by centrifugation and resuspended in a solution capable of inducing competence in *E. coli*. In a more preferred embodiment, *E. coli* DH5 α bacteria containing F' genetic material capable of an enhanced transformation efficiency are harvested by centrifugation at 4°C for 10 minutes at 4,000 rpm and resuspended in FSB (10 mM potassium acetate, 100mM potassium chloride, 45 mM manganese (II) chloride tetrahydrate, 10 mM calcium chloride dihydrate, 3 mM hexamminecobalt (III) chloride, 10% (volume:volume) glycerol, 5% (weight:volume) sucrose, pH 6.4). In an even more preferred embodiment, the re-suspended bacteria containing F' genetic material capable of an enhanced transformation efficiency are subsequently treated with DMSO and can be stored at 70°C for up to 1 year.

In a preferred embodiment, the bacteria are thawed on ice, mixed with exogenous DNA, incubated on ice, and heat treated. In a more preferred embodiment, the frozen *E. coli* DH5 α bacteria containing F' genetic material are thawed on ice for 10 to 15 minutes, mixed with exogenous DNA, incubated on ice for 30 minutes, and heat treated at 42°C for 45 seconds.

In another embodiment of the present invention, the bacteria are treated with high voltage electric pulses in a solution containing exogenous DNA. In a more preferred alternative embodiment of the present invention, the bacteria are *E. coli* DH5 α containing F' genetic material, and are mixed with exogenous DNA and then treated with a brief voltage gradient of 4,000 to 16,000 V/cm.

In a preferred embodiment, high efficiency transformation is preferably 10^8 or greater transformed bacteria per μ g of purified plasmid DNA. High efficiency transformation is more preferably 10^9 or greater transformed bacteria per μ g of purified plasmid DNA.

The present invention also concerns novel *E. coli* bacteria capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of F' episome genetic material. As used herein, such efficiency is said to be "enhanced" if the presence of F' episome genetic material increases the efficiency of transformation of a bacterium. In the preferred embodiment the efficiency of the present invention is enhanced by a factor of greater than one. In a more preferred embodiment, the transformation efficiency of the present invention is enhanced by 2-4 fold. In a even more preferred embodiment, the transformation efficiency of the present invention is enhanced by greater than 4 fold.

The present invention concerns a novel *E. coli* bacterium capable of high efficiency transformation, where said transformation alters the bacterium's genotype and/or transiently alters the bacterium's phenotype. The genotype of an organism is the genetic constitution of an organism. The phenotype of the organism are the characteristics of an organism.

The present invention concerns novel *E. coli* bacteria capable of high efficiency transformation, whose efficiency of transformation is enhanced by the introduction of certain F' episome genetic material. In a preferred embodiment, the bacteria contains all or part of the F' episome genetic material integrated into the *E. coli* chromosome. In another preferred embodiment, the bacteria contains all or part of the F' episome genetic material on a self replicating DNA molecule. In a more preferred embodiment, all or part of the F' episome genetic material is genetically linked to a selectable marker. In an even more preferred embodiment, the F' episome genetic material is linked to an selectable marker providing resistance to an antibiotic, such as a gene providing resistance to tetracycline. In the most preferred embodiment, the F' episome genetic material is derived from the F' episome of XL1-Blue.

The present invention also concerns processes for constructing *E. coli* bacteria containing F' genetic material capable of enhanced transformation

efficiencies. In a preferred embodiment, the bacteria of the current invention is obtained by introducing F' genetic material into an *E. coli* bacterium. In a more preferred embodiment of the present invention, the bacterium of the present invention is obtained by mating *E. coli* XL1-Blue
5 and *E. coli* DH5 α bacteria. In an even more preferred embodiment of the present invention, the bacteria of the present invention is obtained by mating *E. coli* XL1-Blue and *E. coli* DH5 α /pCM301 Δ bacteria (Tucker *et al. Cell* 36: 191-201 (1984)).

10 Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention unless specified.

EXAMPLE 1

Construction of the DH5 α F'1B Strain

15 XL1-Blue and XL2-Blue *E. coli* strains can be obtained from Stratagene (11011 N. Torrey Pines Road, La Jolla, California 92037). DH5 α *E. coli* strains can be obtained from Life Technologies, Inc., P.O. Box 68, Grand Island, NY 14072-0068. To distinguish between the genotypes of DH5 α , XL1-Blue and XL2-Blue *E. coli* strains, DH5 α is transformed with a
20 temperature-sensitive plasmid pCM301 Δ that exhibits ampicillin resistance. *E. coli* strains DH5 α /pCM301 Δ , XL1-Blue and XL2-Blue are then streaked out on LB agar plates (LB agar plates: 15 grams per liter bacto-agar in Lennox LB Broth (10 grams tryptone, 5 grams yeast extract, 5 grams NaCl per liter)) containing appropriate antibiotics. The XL1-Blue and XL2-Blue
25 strains are streaked out on LB plates that contained 15 μ g/ml tetracycline whereas the DH5 α /pCM301 Δ bacteria are streaked out on LB plates

containing 100 µg/ml ampicillin. The plates are incubated overnight at 30°C.

Individual colonies from each strain are isolated and placed into 1 ml of LB medium and grown at 30°C, without shaking, for 4 hours. After
5 the 4 hours, two matings of the *E. coli* strains are set up by mixing 0.5 ml of the DH5α/pCM301Δ culture with 0.1 ml of the XL1-Blue or XL2-Blue culture and 0.5 ml of fresh Lennox LB Broth medium in a 15 ml culture tube that is incubated for 30 minutes at 30°C. Post-mating, the bacteria are diluted either 10⁻² or 10⁻⁴ with Lennox LB Broth and 100 µl of the resultant
10 solution is applied to LB plates containing 100 µg/ml ampicillin and 15 µg/ml tetracycline. These plates are incubated for between 16 to 18 hours at 30°C. Ten individual colonies are selected from the plates and restreaked for single colony isolation on LB AMP₁₀₀ TET₁₅ plates (LB agar plates containing 100 µg/ml ampicillin and 15 µg/ml tetracycline), which
15 are then incubated overnight at 30°C. The colonies are purified through two additional rounds of restreaking on LB AMP₁₀₀ TET₁₅ plates grown at 30°C overnight. The resulting colonies can be designated DH5α F'1 and F'2 corresponding to products from the DH5α/pCM301Δ/XL1-Blue mating and the DH5α/pCM301Δ/XL2-Blue respectively. The temperature
20 sensitive pCM301Δ plasmids are eliminated (cured) from the DH5α F'1 and DH5α F'2 isolates by growing them at 42°C in a shaking incubator (250 rpm) for 16 to 18 hours in 1.5 ml of LB containing 15 µg/ml tetracycline. The cured bacteria are purified by streaking for single colonies on LB TET₁₅ plates and incubated at 37°C for 16 to 18 hours. Individual clones are
25 further purified by restreaking on LB TET₁₅ plates and incubated for an additional 16 to 18 hours at 37°C. The genotype of the DH5α F'1 and DH5α F'2 isolates are confirmed by attempting to grow the isolates on LB AMP₁₀₀ plates and by growing the isolates on LB X-gal IPTG plates (LB agar plates containing 100µg/ml X-gal and 40µg/ml IPTG).

EXAMPLE 2

Production of Competent Bacteria

Working seeds of the DH5 α F'1, DH5 α F'2, XL1-Blue, XL2-Blue and DH5 α strains are generated from single colonies cycled at 28°C for three days. Cycling single colonies involves isolating a single colony for each restreaked strain and restreaking the isolating colony on the appropriate media. The colonies are then grown for 16-18 hours at 28°C. From these plates a single isolated colony from each strain is selected and then restreaked on the appropriate media and grown for 16-18 hours at 28°C. The process of growing the colonies for 16-18 hours at 28°C is repeated three times to acclimate the strain to the growth temperature.

Individual colonies are diluted in 1.5 ml of SOB media. 900 μ l of the diluted colonies are used to inoculate 50 ml of SOB media with 15 μ g/ml tetracycline contained in a 500 ml side baffled flask. The bacteria are then grown at 28°C in a shaking incubator (250 rpm) until the optical density of the solutions is between 0.5 to 0.7 OD₅₅₀ (about 8 to 10 hours). Seeds are produced by mixing a 10 ml sample from the culture with 10 ml of SOB:glycerol (60:40) in a 50 ml tube. The mixture is then incubated on ice for 10 minutes. Aliquots (0.5ml) of the seeds are distributed into 1.2 ml Nunc cryotubes and frozen for 5 minutes in a dry ice:ethanol bath. Seeds are stored at -70°C and used for up to 1 year.

Competent bacteria are produced using a modification of the procedure developed by Hanahan (*J. Mol. Biol.* 166: 557-580 (1983)). A seed culture of each strain (DH5 α F', XL1-Blue, XL2-Blue and DH5 α) are thawed at room temperature and diluted in a ratio of 1:100 with 1 ml SOB containing 20 mM Mg⁺⁺. Aliquots (0.25ml) of the diluted seed are used to inoculate 2.8 liter baffled Fernbach flasks that contained 1.7 L of SOB/20 mM Mg⁺⁺/100 μ g/ml tetracycline. The cultures are incubated at 28°C in a shaking incubator (275 rpm), until they reach an OD₅₅₀ of 0.5. The bacteria

are then harvested by centrifugation in 250 ml Corning centrifuge tubes at 4000 rpm for 10 minutes at 4°C (2300g). The supernatant is then drained from the tubes and the bacteria are resuspended in 20 ml FSB + 5% sucrose (10mM potassium acetate, 100mM potassium chloride, 45 mM manganese (II) chloride tetrahydrate, 10 mM calcium chloride dihydrate, 3 mM hexamminecobalt (III) chloride, 10% (volume:volume) glycerol, 5% (weight:volume) sucrose, pH 6.4). The resuspended bacteria are incubated on ice for 15 minutes. Competence is enhanced by two sequential additions of 0.7 ml DMSO with a 10 minute incubation on ice between additions. After the DMSO additions, the competent bacteria are further incubated on ice for 5 minutes. The competent bacteria are then frozen for 5 minutes in 1.2 ml Nunc Cryotubes containing 0.25 ml of bacteria in a dry ice:ethanol bath. Frozen competent bacteria are stored at -70°C and are stable for up to 1 year.

15

EXAMPLE 3

Transformation Procedures

Competent bacteria are removed from -70°C freezer and thawed on wet ice for about 10 to 15 minutes. Immediately after thawing, the bacteria are gently transferred into pre-chilled polypropylene tubes and gently agitated. Transformation efficiencies, of the competent bacteria are determined by mixing 5 µl (0.05 ng) of control pUC19 DNA to each tube of competent bacteria and incubating the bacteria on ice for 30 minutes. A heat-shock of the bacteria is carried out at 42°C for 45 seconds in water bath. Care is taken not to shake the bacteria during the period of the time the bacteria are undergoing heat treatment. The transformation mixtures are then placed on ice for 2 minutes prior to the addition of 0.9 ml of room temperature SOC (SOC medium is identical to SOB medium except that it contains 20mM glucose) to the transformation mix. The transformation mixtures are incubated at 37°C for 1 hour in a shaker (225 rpm). The transformation reactions are then diluted in a ratio of 1:100 with SOC

medium and 50 μ l or 100 μ l of the diluted samples are spread on LB plates with 100 μ g/ml ampicillin. The plates are then incubated overnight at 37°C.

5 The total transformants per reaction are determined by adding 25 ng of control pUC19 DNA to each 17 x 100 mm polypropylene tube of competent bacteria. The DNA are added by moving the pipette through the bacteria and gently taping the bottom of the tube. The bacteria are incubated on ice for 30 minutes and heat-shocked at 42°C for 45 seconds in water bath. Care is taken not to shake the bacteria during the period of the
10 time the bacteria are undergoing heat treatment. The transformation mix is placed on ice for 2 minutes. After two minutes, 0.9 ml of room temperature SOC is added to the transformation mixture and incubated at 37°C for 1 hour in a shaker (225 rpm). The transformation reactions are diluted in a ratio of 1:10,000 with SOC medium and then 100 μ l of this dilution is
15 spread on LB or YT plates that contained 100 μ g /ml ampicillin. The plates are incubated overnight at 37°C.

A viable cell count of the sample is determined by diluting the bacteria in SOC to 10^{-7} and then spreading 100 μ l from each dilution on LB plates. These plates are incubated overnight at 37°C.

20

| Sample | Transformation Efficiency (transformants/ μ g) | Viable Bacteria | Total Transformants |
|-----------------|---|-------------------|---------------------|
| MAX EFFICIENCY | | | |
| DH5 α | 1.1×10^9 | 1.3×10^9 | 7.7×10^6 |
| DH5 α | 1.4×10^9 | 1.4×10^9 | 4.5×10^6 |
| DH5 α F1 | 6.9×10^9 | 2.5×10^9 | 17×10^6 |
| DH5 α F2 | 6.9×10^9 | 3.1×10^9 | 21×10^6 |
| XL1-Blue | 3.2×10^9 | 2.4×10^9 | 11×10^6 |
| XL2-Blue | 4.3×10^9 | 2.1×10^9 | 15×10^6 |

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as
5 come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

What is claimed is:

1. A bacterium containing F' genetic material capable of an enhanced transformation efficiency.
2. The bacterium according to claim 1, wherein said bacterium is an
5 Enterobacteriaceae.
3. The bacterium according to claim 2, wherein said bacterium is of the genus *Escherichia*.
4. The bacterium according to claim 3, wherein said bacterium is an *Escherichia coli* (*E.coli*).
- 10 5. The *E. coli* bacterium according to claim 4, wherein said bacterium is DH5 α or a derivative thereof or an equivalent thereof.
6. The *E. coli* bacterium according to claim 5, wherein said bacterium is DH5 α .
7. The *E. coli* bacterium according to claim 4, wherein said bacterium
15 contains an F' plasmid.
8. The *E. coli* bacterium according to claim 4, wherein said bacterium contains F' genetic material integrated in the bacterium's genome.
9. The *E. coli* bacterium according to claim 5, wherein said bacterium contains an F' plasmid.
- 20 10. The *E. coli* bacterium according to claim 5, wherein said bacterium contains F' genetic material integrated in the bacterium.
11. The *E. coli* bacterium according to claim 4, further comprising exogenous DNA that replicates independently of the bacterial chromosome.

12. The *E. coli* bacterium according to claim 11, further comprising plasmid DNA.
13. The *E. coli* bacterium according to claim 10, further comprising lambda DNA.
- 5 14. The *E. coli* bacterium according to claim 4, wherein the F' genetic material is derived from the F' genetic material of XL1-Blue.
15. An *E. coli* bacterium according to claim 4, wherein the F' genetic material is derived from the F' genetic material of XL2-Blue.
- 10 16. A process for producing a bacterium capable of an enhanced transformation efficiency, comprising the following steps:
- (a) introducing F' genetic material into a bacterium;
 - (b) selecting said bacterium containing said F' genetic material; and
 - (c) recovering said bacterium containing F' genetic
- 15 material.
17. The process according to claim 16, wherein said bacterium is of the genus *Escherichia*.
18. The process according to claim 17, wherein said bacterium is an *Escherichia coli* (*E.coli*).
- 20 19. The process according to claim 18, wherein said bacterium is DH5 α or a derivative thereof or an equivalent thereof.
20. The process according to claim 19, wherein said bacterium is DH5 α .
21. A process for preparing competent bacteria comprising the following steps:

- (a) growing a bacterium containing F' genetic material capable of an enhanced transformation efficiency in a growth-conductive medium; and
- (b) rendering said bacterium competent.

- 5 22. The process according to claim 21, wherein said bacterium is of the genus *Escherichia*.
23. The process according to claim 22, wherein said bacterium is an *Escherichia coli* (*E.coli*).
24. The process according to claim 23, wherein said bacterium is DH5 α or a derivative thereof or an equivalent thereof.
- 10 25. The process according to claim 24, wherein said bacterium is DH5 α .
26. A preparation of competent bacteria, wherein said preparation is produced by the process comprising:
- (a) growing a bacterium containing F' genetic material
 - 15 capable of an enhanced transformation efficiency in a growth-conductive medium; and
 - (b) rendering said bacterium competent.
27. The preparation according to claim 26, wherein said bacterium is of the genus *Escherichia*.
- 20 28. The preparation according to claim 27, wherein said bacterium is an *Escherichia coli* (*E.coli*).
29. The preparation according to claim 28, wherein said bacterium is DH5 α or a derivative thereof or an equivalent thereof.
30. The preparation according to claim 29, wherein said bacterium is
- 25 DH5 α .

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/01788

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N1/21 C12N15/10 //(C12N1/21,C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|--|
| X | FOCUS, vol. 9, no. 3, 1987, GAITHERSBURG, US, page 13 XP002033496 LISS LR: "New M13 Host: DH5alphaF" competent cells" see the whole document --- | 1-7,9, 11-30 |
| X | J. MOL. BIOL., vol. 166, no. 4, 1983, LONDON UK, pages 557-580, XP000675082 HANAHAN D: "Studies on transformation of Escherichia coli with plasmids" cited in the application see page 559 see page 574 --- -/-- | 1-4,7,9, 11, 14-18, 21-23, 26-28 |

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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INTERNATIONAL SEARCH REPORT

Int. .onal Application No
PCT/US 97/01788

| C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|--|---|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | <p>BIOTECHNIQUES, vol. 5, no. 4, 1987, NATICK US, pages 376-378, XP000675447 BULLOCK WO ET AL.: "XL1-Blue: A high efficiency plasmid transforming recA Escherichia coli strain with beta-galactosidase selection" cited in the application see page 377; figure 1; table 1 -----</p> | <p>1-4,7,9, 11, 14-18, 21-23, 26-28</p> |